THE EFFECTS OF CERTAIN NEUROHUMORS AND OF OTHER DRUGS ON THE VENTRICLE AND RADULA PROTRACTOR OF BUSYCON CANALICULATUM AND ON THE VENTRICLE OF STROMBUS GIGAS [1, 2]

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Krijgsman and Divaris (1955) called attention to the need for pharmacological information about the heart of *Busycon canaliculatum*. Such information was gained in the course of investigations of the physiology of *Busycon*, carried out between 1953 and 1956, and is presented here. For the sake of comparison, experiments on the ventricle of *Busycon* were repeated on the ventricle of *Strombus gigas*. Further experiments with the *Strombus* heart are also reported here. The *Busycon* radula protractor (recommended for physiological investigation by Herrick, 1906) was used for a comparison of the effects of the same drugs on non-cardiac muscle.

I wish to thank Professor John H. Welsh for the suggestion which led to this study and for his guidance.

Pharmacology of the ventricle

Methods

The amplitude of heart beat was measured on kymograph records from isolated ventricles, perfused with sea water through the auricle in a manner similar to that described by Welsh and Smith (1949) for larger crustacean hearts. The bath was so arranged that it could be flushed with sea water while the ventricle was washed through the cannula between tests. Drugs in sea water solution were applied by substitution for the perfusion fluid. Experiments on the *Busycon* ventricle were carried out at room temperature of 23° C., and experiments on the *Strombus* ventricle were carried out at room temperature which varied between 20° and 25° C.

Results

Acetylcholine produced a decrease in amplitude of beat in the *Busycon* ventricle at a 10^{-9} molar concentration, with diastolic arrest at 10^{-7} molar (Fig. 1, A). The

[1] This investigation was supported in part by a predoctoral fellowship, HF-5211, from the National Heart Institute, Public Health Service.

[2] This paper, in slightly different form, comprised part of a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Harvard University.

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[4] Contribution Number 249 from the Bermuda Biological Station. Assisted by a National Science Foundation grant-in-aid through the Bermuda Biological Station.

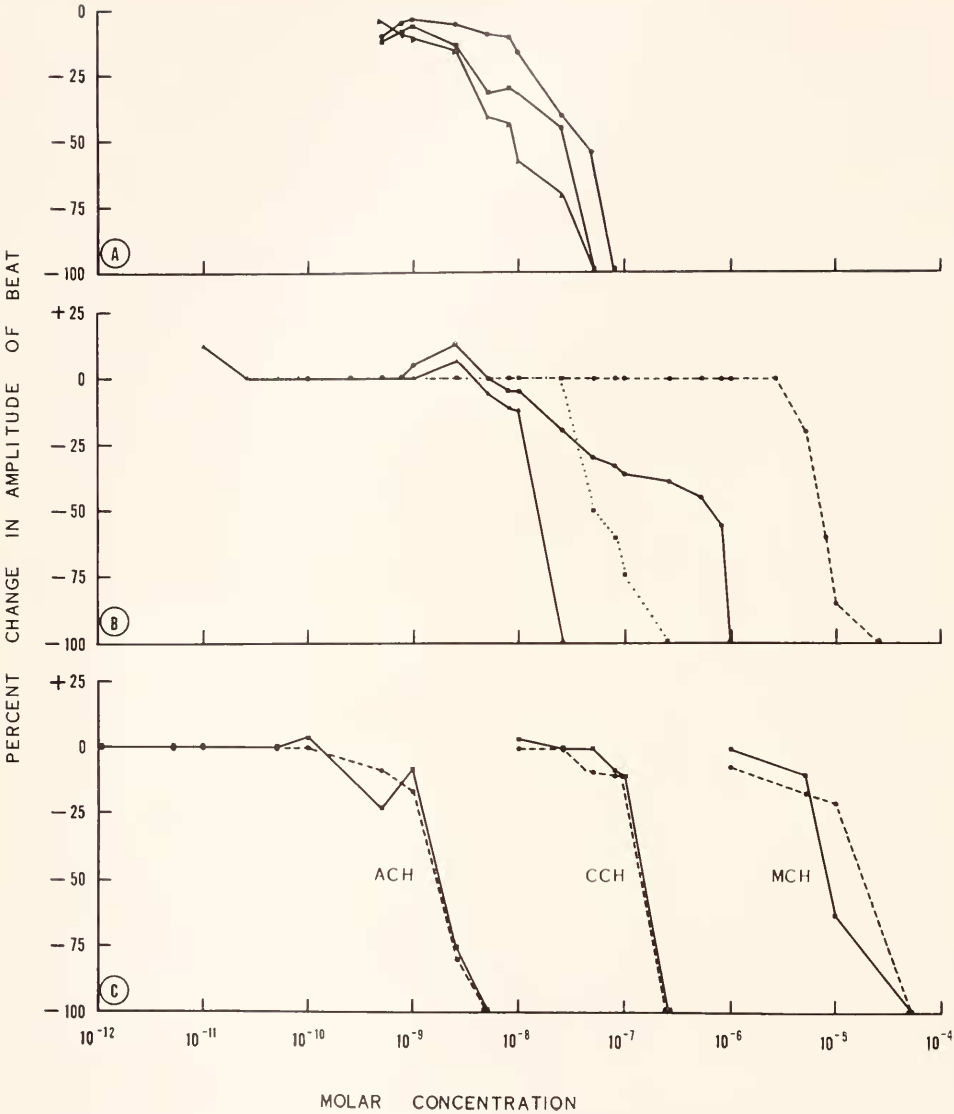

FIGURE 1. A. The effect of acetylcholine on three *Busycon canaliculatum* ventricles. Each point represents the average of the responses of a ventricle to two exposures to the same concentration, once in an ascending series, and once in a descending series. B. The effect of acetylcholine (solid lines), carbamylcholine (dotted line), and acetyl-beta-methylcholine (broken line), on the *Strombus gigas* ventricle. C. The effect of eserine on the concentration-action curve of the *Strombus gigas* ventricle for acetylcholine (ACH), carbamylcholine (CCH), and acetyl-beta-methylcholine (MCH). In each case the solid line represents the effect of the ACH or ACH analogue in the perfusion sea water of an uneserinized heart. The broken line represents the effect of the ACH or ACH analogue applied in perfusion fluid, consisting of a 10^{-5} molar sea water solution of eserine, to a ventricle previously soaked for an hour in 10^{-5} molar eserine.

threshold to acetylcholine was found to be approximately the same for *Strombus,* but the response to a concentration just above threshold was an increase in amplitude, with a decrease in amplitude elicited by concentration ten times threshold (Fig. 1, B). In both *Strombus* and *Busycon,* acetylcholine improved irregular beating at concentrations ten times less than the level of the threshold for an effect on amplitude.

Although carbamylcholine in low concentrations failed to produce the increase in amplitude of the *Strombus* ventricle beat that was seen with acetylcholine, it produced decrease in amplitude in the neighborhood of 5×10^{-8} molar concentration (Fig. 1, B).

Acetyl-beta-methylcholine has an acetylcholine-like effect on the *Strombus* ventricle but with a threshold concentration one thousand times greater (Fig. 1, B).

Eserine failed to potentiate the action of acetylcholine, carbamylcholine, or acetyl-beta-methylcholine on the *Strombus* ventricle. It did regularly abolish the excitatory effect of low concentrations of acetylcholine (Fig. 1, C).

Both adrenalin and noradrenalin proved to have a positive tonotropic effect on the *Busycon* ventricle but the effective concentrations were not in the extremely dilute range at which acetylcholine became effective. At a 10^{-5} molar concentration either neurohumor increased the amplitude of beat about fifty per cent, but the amplitude was increased nearly one hundred per cent when a 10^{-5} molar concentration was obtained as the sum of the molarities of adrenalin and noradrenalin added simultaneously (Fig. 2, A).

5-Hydroxytryptamine was found to have an action on the *Busycon* heart similar to that of adrenalin but with a threshold in the vicinity of 10^{-9} molar, and is thus active in dilutions comparable to acetylcholine dilutions. The *Busycon* ventricle is a thousand times less sensitive to tryptamine than to 5-hydroxytryptamine (Fig. 2, C). Adrenalin or noradrenalin concentrations fifty times greater than threshold concentration for a particular heart irreversibly stop the heart, but 5-hydroxytryptamine at 10^{-2} molar, ten million times the threshold concentration, does not even produce systolic arrest.

In contrast to the synergistic effect of simultaneous addition of adrenalin and noradrenalin to the perfusion fluid, when adrenalin and tryptamine are added to the perfusion fluid simultaneously the effect is not significantly greater than if the same molar concentration were made up of one drug (Fig. 2, B).

5-Hydroxytryptamine acts on the *Strombus* ventricle over a wide range of concentrations with a threshold at 10^{-10} molar (Fig. 3, A).

The antagonism between the negative tonotropic effect of acetylcholine and its analogs, and the positive tonotropic effect of 5-hydroxytryptamine, on the *Strombus* ventricle, is plotted in Figure 3, B, in terms of the reduction by acetylcholine, carbamylcholine, or acetyl-beta-methylcholine of the amplitude maintained by 10^{-7} molar 5-hydroxytryptamine. Acetylcholine acts on the 5-hydroxytryptamine excited ventricle much as on the normal ventricle, but carbamylcholine, which is less effective than acetylcholine in depressing the spontaneous heart beat, is almost as effective an antagonist of 5-hydroxytryptamine as is acetylcholine. Gramine is another antagonist of the action of 5-hydroxptryptamine on the *Strombus* ventricle, and will completely block the action of 10^{-7} molar 5-hydroxytryptamine at a 5×10^{-5} molar gramine concentration.

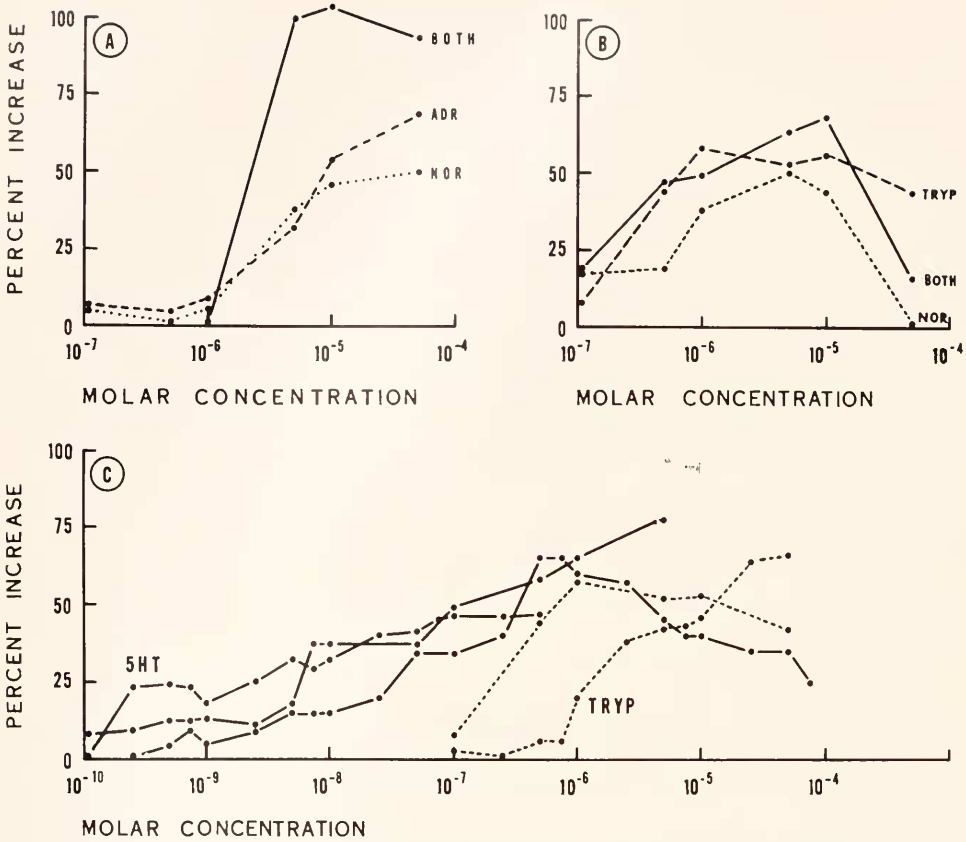

FIGURE 2. A. The effects of adrenalin, of noradrenalin, and of the same molar concentrations made up with equal amounts of the two amines, on the amplitude of beat of the isolated *Busycon canaliculatum* ventricle. Each curve ends at the concentration at which the ventricle stopped in systole. B. A similar comparison of the effects of tryptamine, of noradrenalin, and of both simultaneously on the *Busycon canaliculatum* ventricle. C. The effects of tryptamine and 5-hydroxytryptamine on the amplitude of beat of the isolated *Busycon canaliculatum* ventricle.

DISCUSSION

The pharmacological relations of the *Busycon canaliculatum* ventricle resemble those of other gastropod hearts. Acetylcholine has been shown to depress the beat of the hearts of the gastropods *Buccinum undatum* and *Cyprina islandica* (Welsh, 1956), *Dolabella auricula* (Ebara, 1955), *Cochlitoma zebra* (Divaris and Krijgsman, 1954), *Helix pomatia* (Jullien and Ripplinger, 1950), and *Murex trunculus* (Jullien and Morin, 1931).

Among the hearts listed above, *Murex trunculus* has been reported by Morin and Jullien (1930) to have a small group of nerve cell bodies near the location where Carlson (1905) reports a ganglion in *Busycon*. However, Divaris and Krijgsman (1954) not only found no nervous elements in the white spot at the *Cochlitoma zebra* ventriculo-aortic junction, but also demonstrated the existence of

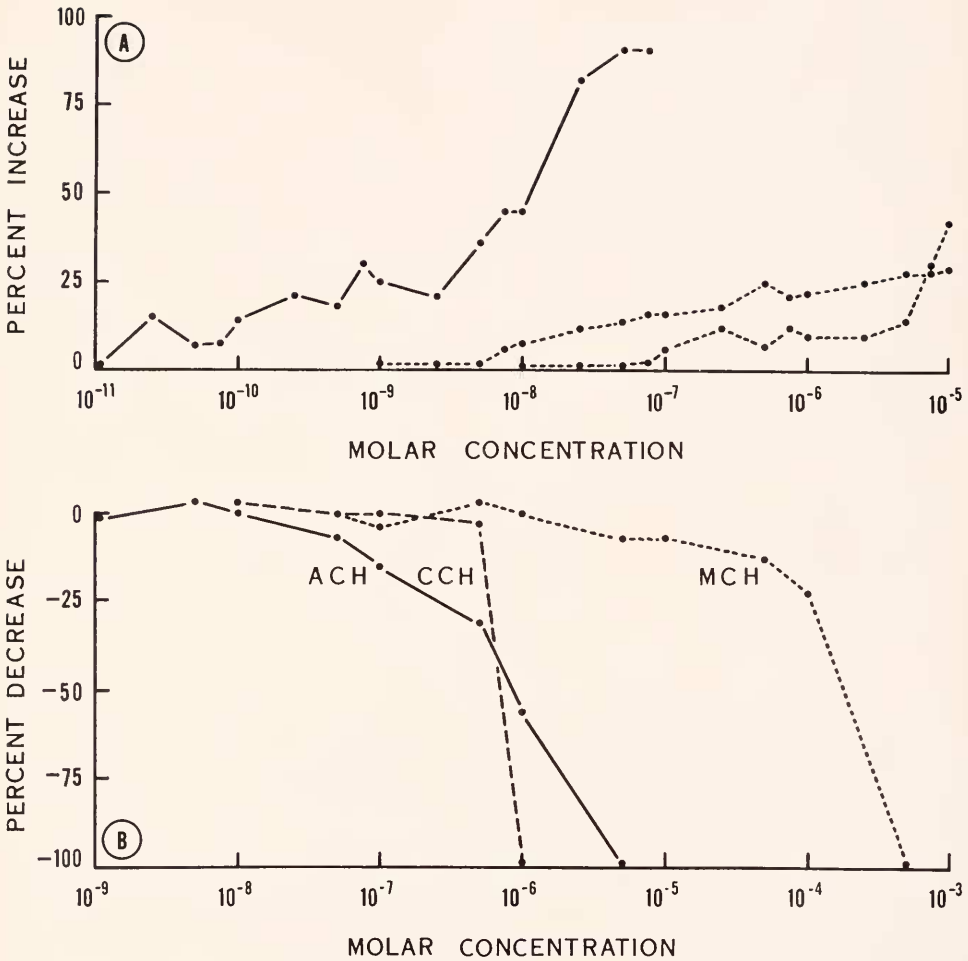

FIGURE 3. A. The effect of 5-hydroxytryptamine on the amplitude of beat of the isolated ventricles of *Strombus gigas* (solid line) and *Aplysia protea* (broken line). B. The effects of acetylcholine, carbamylcholine, and acetylbetamethyl choline, on the amplitude of beat, of the *Strombus gigas* ventricle, which is maintained by perfusion with 10^{-7} molar 5-hydroxytryptamine.

myogenic pacemakers. A myogenic origin for the beat of the heart of *Murex trunculus* was indicated when Cardot, Jullien and Morin (1929) showed that isolated fragments would beat in sea water. Thus, the *Busycon* ventricle reacts to acetylcholine like the hearts of other gastropods which have been demonstrated to have myogenic pacemakers.

The extremely low concentrations at which acetylcholine and 5-hydroxytryptamine are effective on the *Busycon* ventricle are in accordance with Welsh's (1957) finding that they act as neurohumors in *Venus mercenaria*. That 5-hydroxytryptamine is effective in lower concentration than adrenalin or noradrenalin, with an equally rapid onset of action, suggests that 5-hydroxytryptamine might

be closer in structure to the natural cardio-regulatory neurohumor of *Busycon* than are the mammalian neurohumors. 5-Hydroxytryptamine has, in fact, been found in the pooled ganglia of *Busycon* by Welsh (1954).

The response of the *Busycon canaliculatum* ventricle to adrenalin and noradrenalin is evidence that it is more similar pharmacologically to the hearts of the molluscs *Eledone cirrosa* and *Anodonta* (Fänge and Ostlund, 1954), which respond by an increase in amplitude of beat, than to that of *Aplysia dactylomela,* which is insensitive to the two neurohumors, as reported by von Euler, Chavez and Teodosio (1952). That the isolated *Busycon* heart beats so well is also a contrast to the *Aplysia dactylomela* heart which will beat spontaneously only if adjacent ganglia are isolated with it (von Euler, Chavez, and Teodosio, 1952). However, I have found the isolated ventricle of *Aplysia* to beat spontaneously, although not well. Its beat may be sustained by ergonovine or by 5-hydroxytryptamine, but its threshold to 5-hydroxtryptamine is between 10^{-5} and 10^{-7}, which is considerably higher than the thresholds of the *Busycon* and *Strombus* ventricles (Fig. 3, A).

Pharmacology of the leached ventricle

Following Burn's theory (1950) of the relation of local hormones to cardiac automatism, it might be expected that a denervated ventricle, which had been deprived, by leaching, of previously synthesized neurohumors, would respond by contraction to either acetylcholine or 5-hydroxytryptamine.

Methods

In order to ascertain the upper limit to the time an isolated *Busycon canaliculatum* ventricle may remain viable and useful for bioassay, six isolated entire hearts were set aside in sea water at 9° C. for periods ranging from one to six weeks. At intervals, a ventricle was removed to room temperature, allowed five hours for adjustment, and then perfused. It would seem probable that after a week the cut distal portions of the cardioregulatory nerves (from the visceral ganglion; Carlson, 1905) would have degenerated, so that the leached ventricle might react to pharmacological agents primarily as a muscle preparation.

Results

Of two hearts kept at 9° C. for one week, both survived and both beat normally when perfused. That is, after one-half hour one was beating at 18 systoles per minute, the other at 21 and both were emptying completely at each systole. Each continued at its original rate for 5 hours, at the end of which time one showed a threshold response to 5×10^{-9} *M* 5-hydroxytryptamine and 10^{-9} *M* acetylcholine, and the other a threshold response to 10^{-9} *M* acetylcholine and to 10^{-9} *M* 5-hydroxytryptamine.

Of the two hearts set aside for three weeks, only one survived. The other, after one-half hour of perfusion, was beating regularly at a beat of 22 systoles per minute. After three hours it had slowed down to 12 systoles per minute but was still beating regularly. Now, however, the ventricle was no longer emptying completely and relaxed to three times its previous volume at diastole. By increasing the pressure of perfusion the rate was increased to 18 systoles per minute and, while the ventricle

retained the full relaxation at diastole, it returned to complete emptying at systole. After eight hours of perfusion, the rate was down to nine systoles per minute but was restored to eighteen by an increase in pressure. After ten hours of perfusion the rate had dropped again to ten systoles per minute and a further increase in pressure was required to raise it to sixteen. After this ventricle, which had been set aside for three weeks in sea water at 9° C., had maintained an uninterrupted rhythm for fourteen hours and sixteen minutes its threshold for 5HT was found to be in the neighborhood of a 5×10^{-9} molar concentration and its threshold for acetylcholine was 10^{-10} molar.

Of the two hearts set aside for six weeks at 9° C., one survived. When set up in a heart bath and perfused with sea water the ventricle showed no sign of spontaneous activity, but it did react to 10^{-7} molar 5HT by beating at the rate of 18 systoles per minute as long as it was subjected to 5HT.

Subsequently, four hearts were taken which had survived four weeks at 11° C. but which did not beat spontaneously when perfused. Each was subjected to concentrations of acetylcholine and of 5-hydroxytryptamine from 10^{-12} to 10^{-2} molar at half-molar intervals. No concentration of acetylcholine provoked beating. All four hearts beat, when perfused with 10^{-7} molar 5-hydroxytryptamine, at a normal rate but at an amplitude much less than that which had been elicited by 5-hydroxytryptamine after soaking at 9° C. for six weeks.

Discussion

The failure of acetylcholine to restore automatism is in accord with the similar findings of Jensen (1957) with several lamellibranch hearts.

Pharmacology of the radula protractor

Methods

The radula protractor was isolated intact, attached to a bit of radula sac at one end, and to a fragment of odontophore at the other, and set up in a sea water bath at 19° to 21° C. Drugs in sea water solution were added to the bath to produce the desired molar concentration. Ejection from a syringe assured thorough mixing. Air was bubbled through the bath from a capillary tube entering at the bottom.

Results

Spontaneous contractions do not occur in a *Busycon canaliculatum* radula protractor isolated and maintained under slight tension in a constant temperature sea water bath. This makes it a more favorable preparation for the study of induced contractions than the more commonly used snail retractor pharyngis, where rhythmicity obscures the effect of stimulation (Masai, 1951).

Acetylcholine at 10^{-5} molar concentration will induce a contraction which reaches full amplitude immediately. When the acetylcholine is washed off, the muscle relaxes immediately. If the acetylcholine solution is left in contact with the muscle, the contraction declines slightly for five or ten minutes, and then the rate of relaxation accelerates, and by forty-five minutes after adding acetylcholine

the muscle is relaxed again. The bathing solution has then lost its ability to contract a fresh muscle. Normal responsiveness of the muscle is restored by ten minutes of washing with aerated sea water.

When 10^{-5} *M* acetylcholine is followed in three minutes by sufficient tryptamine to produce a concentration in the bath of 10^{-3} *M*, relaxation is not an immediate fall in tension such as is seen when acetylcholine is washed off, but is gradual at first and then develops into rhythmic pulsation with a slowly declining base line (Fig. 4, A). The rhythmicity may be as regular in rate and amplitude as a heartbeat and ceases abruptly with the relaxation that follows when the mixture of the two drugs is washed off. In each of the rhythmic contractions the radula protractor shortened to about one-third of its resting length. Figure 4, B, shows the similar response when the muscle is subjected first to 10^{-5} *M* acetylcholine and then in two minutes to the combination of 10^{-5}*M* acetylcholine and 10^{-3} *M* 5-hydroxy-

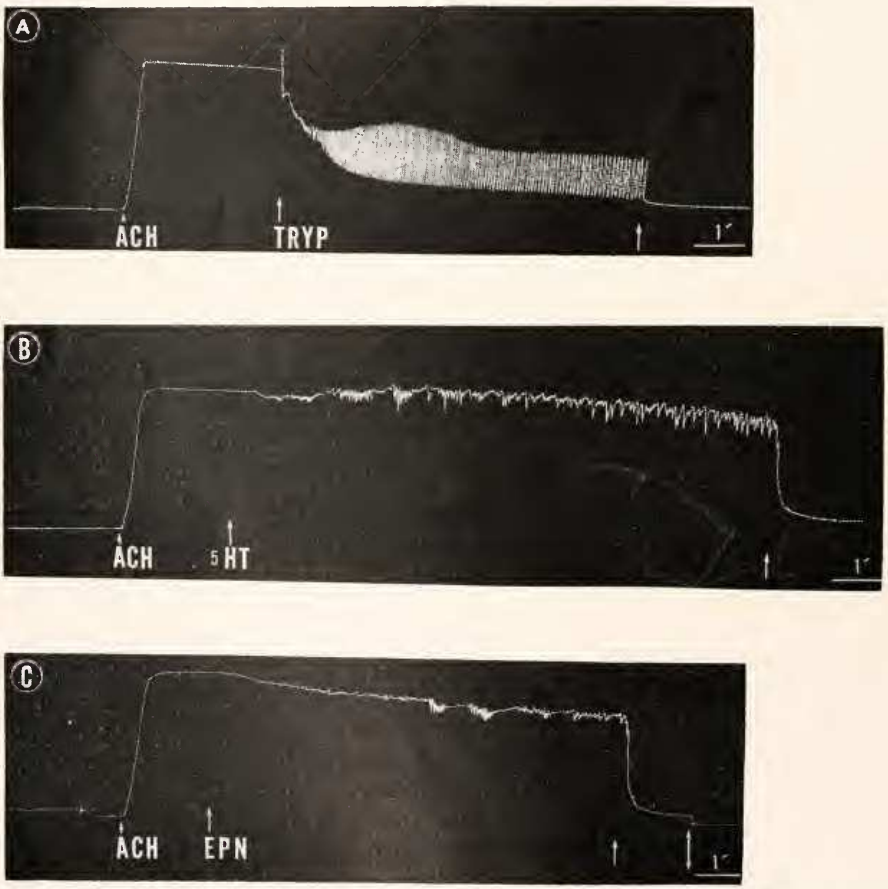

FIGURE 4. Responses of the *Busycon canaliculatum* radula protractor to 10^{-5} molar acetylcholine (ACH) followed by 10^{-3} molar tryptamine (TRYP), 5-hydroxytryptamine (5-HT), or adrenalin (EPN).

tryptamine. Figure 4, C, shows the response obtained when the radula protractor is subjected first to 10^{-5} *M* acetylcholine and then in two minutes to 10^{-3} *M* adrenalin, also. These combinations were found to be optimum for regularity and amplitude of "beat," and it may be seen that tryptamine was the most effective of the three amines.

Figure 5, A, shows that in the radula retractor, tryptamine following acetylcholine produces a similar rhythmic decline of tension. The same is true for the odontophore retractor (Fig. 5, B). It may be seen that in Figure 5, A, the first effect of tryptamine was a slight decline in tension while in Figure 5, B, it was a slight increase. The first effect seems to vary randomly for all three radula apparatus muscles, but is always followed by the rhythmic relaxation.

In Figure 5, A, at T_1, 10^{-5} *M* tryptamine was added in the absence of prior stimulation by acetylcholine, and at T_2, the concentration was brought to 10^{-4} *M* tryptamine. Neither contraction nor relaxation was elicited, yet at the second T_2 the same concentration (following acetylcholine) produced relaxation. At the temperature of these experiments, 19-21° C., no concentration of tryptamine, 5-hydroxytryptamine, or adrenalin relaxed a radula apparatus muscle that had not been previously excited to contract. Later it was found that at 27° C. 5-hydroxytryptamine would cause a previously unstimulated muscle to contract slowly and irregularly, but never to relax.

A radula protractor in 10^{-3} *M* 5-hydroxytryptamine develops a sensitivity to stretching not shown in the muscle simply isolated in sea water. It responds to a sharp tug by a quick contraction followed by slower relaxation.

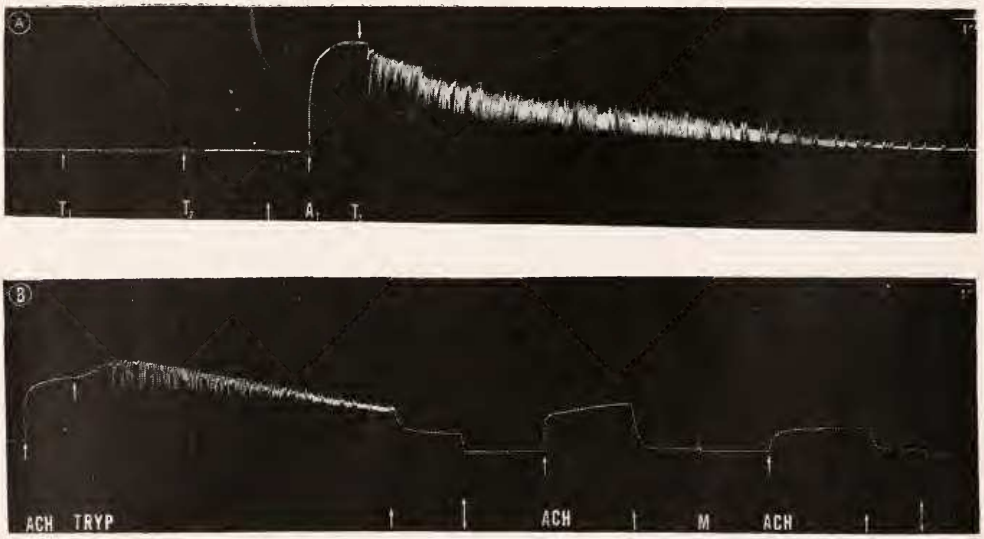

FIGURE 5. *Busycon canaliculatum*. A. Radula retractor: $T_1 = 10^{-5}$ molar tryptamine, $T_2 = 10^{-4}$ molar tryptamine, $A_1 = 10^{-5}$ molar acetylcholine. B. Odontophore retractor: ACH = 10^{-4} molar acetylcholine, TRYP = 10^{-3} molar tryptamine, M = 10^{-5} molar Mytolon for a half hour. A plain arrow indicates that the muscle was washed with sea water with the drum moving, and a double-ended arrow indicates that the muscle was washed with sea water for an hour with the drum stopped.

Acetylcholine contraction of the radula protractor may be blocked both by an agent active at motor end plates, d-tubocurarine, and by the most effective antagonist of acetylcholine on the *Venus mercenaria* heart (Luduena and Brown, 1952), Mytolon. When 10^{-3} molar d-tubocurarine is applied for half an hour the contraction elicited by 10^{-5} molar acetylcholine is blocked but not that due to 0.5% KH_2PO_4. Similarly, 1:10,000 Mytolon applied for a half hour greatly reduces the contraction elicited by 10^{-5} molar acetylcholine but has no effect on the contraction following 0.5% KH_2PO_4.

Acetylcholine contraction of the radula protractor is potentiated by eserine. When the graded responses of the same muscle to an increasing series of acetylcholine concentrations, before and after soaking for an hour in 1:10,000 eserine, are compared, it is found that the response at each concentration is augmented although the threshold to acetylcholine is not altered. Prior soaking in eserine has the same effect on the rhythmicity obtained with acetylcholine and tryptamine as has increasing the concentration of acetylcholine used.

Lysergic acid diethylamide is antagonistic toward the production of a "beat" by the combined action of acetylcholine and tryptamine, but does not itself cause contraction or relaxation at concentrations from 10^{-5} molar to 10^{-10} molar.

Discussion

The rhythmic "beat" of the radula protractor suggests a model of the heart beat. Acetylcholine and 5-hydroxytryptamine both occur as natural neurohumors, both will regulate the *Busycon* heart, and together they induce a rhythmicity in the *Busycon* radula protractor comparable to the automatic rhythmicity of the heart. Tryptamine, however, is more effective than 5-hydroxytryptamine in inducing rhythmicity, whereas 5-hydroxytryptamine is more effective on the heart. Heart strips, when cut to dimensions similar to the radula protractor and set up on the same apparatus, respond to 5-hydroxytryptamine with rhythmic contractions, which are opposed by acetylcholine.

It is tempting to speculate that the radula protractor "beat" might originate in the presence at the cell surface of the opposing neurohumors in the right proportions for alternate action. Welsh and Slocombe (1952) suggest that released acetylcholine depresses the *Venus mercenaria* heart by changing the membrane polarization of muscle fibers and thus interfering with normal contraction and the normal spread of excitation. The effects of acetylcholine and 5-hydroxytryptamine on the surface membrane polarity of a non-cardiac molluscan smooth muscle have been investigated by Twarog (1954). She found that acetylcholine depolarized the *Mytilus edulis* anterior byssus retractor and initiated contraction. 5-Hydroxytryptamine caused immediate relaxation but produced no change in membrane polarization. Furthermore, when the acetylcholine was washed off, the muscle immediately repolarized, but the contraction persisted. (It may be recalled that when acetylcholine was washed off the radula protractor, the muscle relaxed immediately). Twarog suggests that it is probable that the depolarization induced by acetylcholine is directly related to the ensuing contraction. The failure of 5-hydroxytryptamine to produce membrane changes while relaxing the muscle could be attributed to a direct action on the contractile element.

A possible explanation for the rhythmicity induced in the radula protractor by

acetylcholine and tryptamine could be based on Twarog's byssus retractor results. It could be supposed that the acetylcholine in the bath kept the muscle cells depolarized, which would lead to contraction. The tryptamine also in the bath would relax the contractile elements and a second contraction would then occur in response to the surface depolarization.

One alternative hypothesis would be that acetylcholine depolarized the muscle fiber surface membrane and that tryptamine then repolarized it. If it is supposed that contraction follows depolarization and relaxation follows repolarization, the "beat" might be explained. That the applied acetylcholine and tryptamine act on muscle rather than on nerve may be indicated by the persistence of susceptibility to induced "beating" in isolated radula protractors stored for a week.

Summary

1. The hearts of *Busycon canaliculatum* and *Strombus gigas* were found to respond to applied neurohumors as do the myogenic hearts of other gastropods. Acetylcholine was cardio-inhibitory, and 5-hydroxytryptamine was cardio-accelera-tory, in concentrations low enough to suggest that they might be the normal regulatory neurohumors.

2. The *Busycon canaliculatum* radula protractor was contracted by acetylcho-line, and could then be relaxed rhythmically by 5-hydroxytryptamine, tryptamine, and adrenalin, all of which raise the tonus of the ventricle.

LITERATURE CITED

Burn, J. H., 1950. Relation of motor and inhibitor effects of local hormones. *Physiol. Rev.,* **30**: 177–193.

Cardot, H., A. Jullien and G. Morin, 1929. Sur l'automatisme de divers lambeaux du coeur de *Murex trunculus* places dans l'eau de mer. *C. R. Soc. Biol., Paris,* **102**: 441.

Carlson, A. J., 1905. Comparative physiology of the invertebrate heart. I. The innervation of the heart. *Biol. Bull.,* **8**: 123–170.

Divaris, C. A., and B. J. Krijgsman, 1954. Investigation into the heart function of *Cochlitoma zebra. Arch. Int. Physiol.,* **62**: 211–233.

Ebara, A., 1955. Physiological studies on the cardiac nerves of a marine mollusc; *Dolabella auricula* Solander. *Sci. Rep. Tokyo Kyoiku Daigaku B,* **7**: 219–230.

von Euler, U. S., N. Chavez and N. Teodosio, 1952. Effects of acetylcholine, noradrenaline, adrenaline, and histamine on isolated organs of *Aplysia* and *Holothuria. Acta Physiol. Latinoam.,* **2**: 101–106.

Fänge, R., and E. Ostlund, 1954. The effects of adrenaline, noradrenaline, tyramine, and other drugs on the isolated heart from marine vertebrates and a cephalopod (*Eledone cirrosa*). *Acta Zool.,* **35**: 289–305.

Herrick, J. C., 1906. Mechanism of the odontophoral apparatus in *Sycotypus canaliculatus. Amer. Nat.,* **40**: 707–737.

Jensen, D., 1957. Rôle of acetylcholine in cardiac automatism. *Fed. Proc.,* **16**: 67.

Jullien, A., and G. Morin, 1931. Action comparée de l'atropine et de l'acétylcholine sur le ventricule isolé de l'escargot et du *Murex. C.R.Soc. Biol., Paris,* **106**: 187–189.

Jullien, A., and J. Ripplinger, 1950. Les excitations faibles du nerf cardiaque d'*Helix pomatia* produisent de l'acétylcholine, sans modifications apparentes du rhythme car-diaque. *C. R. Soc. Biol., Paris,* **144**: 544–545.

Krijgsman, B. J., and G. A. Divaris, 1955. Contractile and pacemaker mechanisms of the heart of molluscs. *Biol. Rev.,* **30**: 1–39.

Luduena, F. P., and T. G. Brown, 1952. Mytolon and related compounds as antagonists of acetylcholine on the heart of *Venus mercenaria. J. Pharm. Exp. Ther.,* **105**: 232–239.

MASAI, S., 1951. Tonic and Phasic contractions of the retractor pharyngis of snail (*Euhadra lubuana*). *Japan. J. Physiol.*, 2: 60–68.

MORIN, G., AND A. JULLIEN, 1930. Sur la structure du coeur chez *Murex trunculus*. *Bull. Hist. App. Physiol. Path. Tech. Micr., Lyon,* 7: 79–96.

RIJLANT, P., 1931. L'automatisme du coeur des Gastéropodes *Fulgur carica* et *Fulgur canaliculatum, Polyniccs*. *C. R. Soc. Biol., Paris,* **108**: 1150–1152.

TWAROG, B. M., 1954. Responses of a molluscan smooth muscle to acetylcholine and 5-hydroxytryptamine. *J. Cell. Comp. Physiol.*, **44**: 141–164.

WELSH, J. H., 1954. Hydroxytryptamine: a neurohormone in the invertebrates. *Fed. Proc.*, **13**: 162–163.

WELSH, J. H., 1956. Neurohormones of invertebrates. I. Cardio-regulators of *Cyprina* and *Buccinum*. *J. Mar. Biol. Assoc.*, **35**: 193–201.

WELSH, J. H., 1957. Serotonin as a possible neurohumoral agent: evidence obtained in lower animals. *Ann. N. Y. Acad. Sci.*, **66**: 618–630.

WELSH, J. H., AND A. G. SLOCOMBE, 1952. The mechanism of action of acetylcholine on the *Venus* heart. *Biol. Bull.*, **102**: 48–57.

WELSH, J. H., AND R. I. SMITH, 1949. Laboratory Exercises in Invertebrate Physiology. Burgess Publishing Company, Minneapolis, Minnesota.